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# Effect of the HIV-1 Syncytium-Inducing Phenotype on Disease Stage in Vertically-Infected Children

**Joseph E. Fitzgibbon,[1]* Sunanda Gaur,[2] Medha Gavai,[2] Patrice Gregory,[3] Lawrence D. Frenkel,[2] and Joseph F. John, Jr.[1]**

[1]*Department of Medicine, Division of Allergy, Immunology, and Infectious Diseases, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, Piscataway, New Jersey*
[2]*Department of Pediatrics, Division of Immunology, Allergy, and Infectious Diseases, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, Piscataway, New Jersey*
[3]*Department of Family Medicine, University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, Piscataway, New Jersey*

The syncytium-inducing (SI) capability of HIV-1 isolates from 48 HIV-infected children was determined in order to examine the association of the SI phenotype with an AIDS diagnosis and/or with other clinical parameters in HIV-infected children. In a retrospective cross-sectional analysis, phenotypic data were linked to clinical and immunologic data from each patient. Multiple longitudinal samples were analyzed from 14 patients. Children with SI viruses were older than children with nonsyncytium-inducing (NSI) strains. Twelve of 13 children less than 2 years old carried NSI viruses, seven of the 12 already had a diagnosis of AIDS. Two children under 2 years of age died within 1 month of NSI virus isolation. Although plasma p24 antigen levels tended to be higher in the NSI group, the difference appeared to reflect high p24 levels in children under 2 years old with AIDS. When children under 2 were omitted, differences in age, CD4+ cell counts, p24 antigenemia, and clinical parameters were not significant. The SI phenotype of HIV-1 did not occur more frequently in children with an AIDS diagnosis. Four children remained stable with SI isolates over time periods of 16 to 31 months. Three children's isolates converted from NSI to SI and 2 converted from SI to NSI. These data indicate that SI viruses do not play a significant role in progression to AIDS during the first 2 years of life. Furthermore, for children above the age of 2, the association between advanced disease stage and the SI phenotype in adults may not apply. ***J. Med. Virol. 55:56–63, 1998.*** 

**KEY WORDS:** pathogenesis; virulence; pediatrics



## INTRODUCTION

HIV-1 clinical isolates can be divided into two groups according to their growth characteristics in vitro. Some isolates grow slowly and to low titers in peripheral blood mononuclear cell (PBMC) cultures. These slow-growing isolates tend to be macrophage-tropic and generally do not grow in transformed T-cell lines. Since they usually lack the ability to induce syncytium formation when grown in MT-2 cells, they are known as "NSI" (nonsyncytium-inducing) strains [Asjo et al., 1986; Evans et al., 1987]. In contrast, other isolates of HIV-1 grow quickly to high titers in PBMC cultures and can infect T-cell lines. These strains usually form easily identifiable syncytia in MT-2 cells and are termed "syncytium-inducing (SI)" viruses [Asjo et al., 1986; Evans et al., 1987]. NSI isolates are typically detected in asymptomatic adult patients relatively early in the course of infection [Tersmette et al., 1988, 1989; Bozzette et al., 1993]. In general, NSI viruses tend to utilize the β-chemokine receptor CCR5 as a second cellular receptor in addition to CD4 [Alkhatib et al., 1996], the primary HIV-1 receptor. Inability to infect T-cell lines is due, in large part, to the absence of CCR5 expression on the surface of these cells [Alkhatib et al., 1996].

In HIV-infected adults, conversion from the NSI to the SI phenotype often precedes a period of more rapid CD4+ cell decline and disease progression [Tersmette et al., 1988, 1989; Koot et al., 1993a; Richman and Bozzette, 1994], although conversion to the SI phenotype in adults is not always a prerequisite for disease pro-

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*Correspondence to: Joseph E. Fitzgibbon, Ph.D., Department of Medicine, Division of Allergy, Immunology, and Infectious Diseases, UMDNJ-Robert Wood Johnson Medical School, 1 Robert Wood Johnson Place, New Brunswick, NJ 08903. E-mail fitzg@umdnj.edu.

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gression, CD4+ cell decline, or death [Koot et al., 1993a]. The switch from NSI to SI in adult patients is accompanied by changes in the envelope glycoprotein of HIV-1 that enable the virus to infect and kill T-cells [Fouchier et al., 1992]. These changes allow the virus to utilize an alternative second receptor CXCR4, which is generally expressed on the surface of T-cells [Deng et al., 1996]. The balance between CD4+ T-cell production by the host and CD4+ T-cell destruction by the virus [Ho et al., 1995; Wei et al., 1995] may be tipped more quickly in favor of the virus after the faster-replicating SI variants develop. Accordingly, in the adult patient, the development of SI variants carries a poor prognosis [Bozzette et al., 1993; Richman and Bozzette, 1994]. Moreover, there is some evidence that adult patients with faster-replicating SI viruses will develop resistance to antiviral drugs such as zidovudine more quickly than those with NSI viruses [Boucher et al., 1992; Koot et al., 1993b].

The impact of the SI phenotype is less clear in pediatric patients. An early study by De Rossi et al. [1993] indicated that most HIV-1 isolates from children in late stages of infection were more likely to be capable of replication to high titers in PBMC's and T-cell lines. Although they did not measure SI capability directly, the "rapid/high" growth characteristics they describe are associated with the SI phenotype in adults. Gupta et al. [1993], in a smaller study involving HIV-1 isolates from 13 pediatric patients, failed to find a correlation between the presence of SI isolates in children and a diagnosis of AIDS. Spencer et al. [1994] demonstrated a biphasic clinical response to HIV-1 infection in children. Disease progression occurred frequently in children less than 1 year old despite the fact that they all carried NSI viruses. However, children who survived the early years tended to develop SI viruses as they got older. In those older children the SI phenotype was associated with lower CD4+ cell counts and a greater risk of zidovudine resistance. Another study [Mammano et al., 1995] did indicate an association between the SI phenotype and disease stage in a group of perinatally-infected children. Thus, previous studies of pediatric patients have yielded conflicting results regarding the significance of the SI phenotype in HIV-infected children.

To help characterize the significance of the SI phenotype in pediatric patients, we correlated the phenotype of HIV-1 isolates from 48 vertically-infected children with various measures of HIV-1 disease including a diagnosis of AIDS, age-adjusted CD4+ cell counts, plasma p24 antigen levels, and the presence of several common AIDS-associated symptoms.

## MATERIALS AND METHODS

### Patient Population

Forty-eight HIV-positive, vertically-infected children followed by the Central New Jersey Pediatric AIDS Program of Robert Wood Johnson Medical School from whom positive HIV-1 cultures had been obtained were included in the study. Informed consent was obtained from the parent or guardian of each subject involved in the study. The age range of study subjects was 4 to 129 months. Most (40 of 48) of the children were enrolled in Pediatric AIDS Clinical Trials Group (PACTG) clinical trials involving only nucleoside analog antivirals. The importance of the SI phenotype in this group of pediatric patients was studied in a cross-sectional fashion. In addition, multiple longitudinal samples were available from 14 of the patients and results obtained from these samples are described. For the cross-sectional study we arbitrarily chose the latest sample from each of these 14 patients for inclusion in the analysis. When data from all children were analyzed using the earliest available sample from each of the of the 14 longitudinally sampled children, the results were not significantly altered (data not shown). All clinical and laboratory data were evaluated with respect to the date of sampling used for the evaluation of SI phenotype.

### Clinical Evaluations

Children were evaluated at each clinic visit when blood samples were obtained for cultures. Clinical data, reviewed retrospectively, included: lymphoid interstitial pneumonitis, serious bacterial infection, encephalopathy, and AIDS; all were defined according to established CDC guidelines [Caldwell et al., 1994]. Opportunistic infections were defined according to established guidelines [Feigin and Matson, 1992]. Patients were considered positive for CMV if a urine culture was positive for CMV prior to the date of sampling for HIV-1 culture. Age-adjusted CD4 cell counts were calculated for each child at or near the time of each sampling. These were expressed as a percentage of the median CD4+ cell count calculated for healthy children in various age groups [Denny et al., 1992].

### Plasma p24 Antigen Assay

Free plasma p24 antigen was measured using an antigen-capture enzyme-linked immunosorbent assay kit (Abbott Laboratories, Abbott Park, IL).

### MT-2 Assay for Syncytium Formation

MT-2 assays were carried out as described by Japour et al. [1994]. Briefly, supernatants (50 μl) from HIV-1 positive virus cultures, performed as previously described [Hollinger et al., 1992], were added to duplicate wells containing 50,000 MT-2 cells [Harada et al., 1985] in 150 μl medium (RPMI-1640 supplemented with 20% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin) in 96-well plates. HIV-1 NL-43 [Adachi et al., 1986] was used as a positive control in each assay, uninoculated wells served as negative controls. Cells were examined microscopically for cytopathology at 3–4 day intervals for a total of 14 days. After each examination one-half the culture volume was removed and replaced with fresh medium. To confirm the infectious potential of each inoculum, duplicate cultures containing 200,000 PHA-stimulated, HIV-negative donor PBMC's were set up and fed along with the MT-2 cultures. The medium used for the PBMC cultures was identical to that used

TABLE I. Clinical Characteristics of Children With NSI vs. SI Viruses

| | SI | NSI | p Value |
|---|---|---|---|
| Number of patients | 15 | 33 | |
| Age (mean in months ± SE) | 64 ± 8 | 41 ± 6 | 0.014[c] |
| CD4+ Count (cells/mm$^3$ blood, mean ± SE) | | | |
| Absolute | 340 ± 107 | 758 ± 154 | 0.039[c] |
| % Median[a] | 24 ± 7 | 41 ± 8 | 0.158[c] |
| Plasma p24[b] | 46 ± 27 | 67 ± 16 | 0.086[c] |
| No. p24 Antigen Positive (%)[f] | 4 (27) | 14 (42) | 0.296[d] |
| Antiviral therapy (mean in weeks ± SE) | 82 ± 16 | 40 ± 8 | 0.018[c] |
| PCP prophylaxis at time of sampling (%) | 12 (80) | 24 (73) | 0.728[e] |
| AIDS Diagnosis (%) | 4 (27) | 16 (49) | 0.155[d] |
| Opportunistic Infection (%) | 3 (20) | 12 (36) | 0.328[e] |
| Encephalopathy (%) | 3 (20) | 12 (36) | 0.328[e] |
| Lymphoid Interstitial Pneumonitis (%) | 3 (20) | 5 (15) | 0.692[e] |
| Bacterial infection (%) | 1 (7) | 0 (0) | 0.313[e] |
| CMV infection (%) | 6 (40) | 13 (39) | 0.968[d] |

[a]Percentage of age-adjusted normal median CD4+ cell count was calculated for each patient, the mean of the values ± SE for the SI and NSI groups is shown.
[b]Plasma p24 data were obtained for 14 of the SI subjects and 29 of the NSI's.
[c]Wilcoxon Rank Sum Test.
[d]Chi-Square Test.
[e]Fisher's Exact Test.
[f]Plasma p24 level greater than 30 pg/ml.

for the MT-2 cultures except that the PBMC medium was supplemented with 3% T-cell growth factor (IL-2). At day 14, supernatants from the PBMC cultures were assayed for reverse transcriptase activity according to previously published procedures [Willey et al., 1988]. All SI isolates were positive for reverse transcriptase activity; isolates were scored as NSI only if the PBMC cultures were positive for reverse transcriptase activity and the MT-2 cultures were negative for cytopathology.

### Statistical Analyses

The data were described using means, standard errors of the mean, and percentages. To compare the differences for continuous variables between NSI and SI groups, Wilcoxon Rank Sum tests were used for the immunologic measures and the patient age. Categorical variables that characterized patient symptoms and outcomes were compared for NSI and SI patients with Chi-square or Fisher's Exact tests. Multiple logistic regression was done to study the relationship between phenotype and development of an AIDS diagnosis while controlling for differences in age, percent of age-adjusted normal median CD4+ cell count, and p24 antigenemia. All tests of significance were two-tailed. Results were considered statistically significant when $P < 0.05$.

## RESULTS

### Cross-Sectional Analysis

Of the 48 children studied, 33 had NSI isolates and 15 had SI isolates. Data obtained from these children are listed in Table I. Children with SI isolates were significantly older than those with NSI isolates. The age range can be best seen in Figure 1. The mean age of the NSI group was 41 ± 6 months vs. 64 ± 8 months for the SI group ($P = 0.014$). Nevertheless, one of the 15 SI isolates was from a child less than 2 years old.

Detectable levels of HIV-1 p24 antigen were present in the plasma of 27% of the children with SI viruses vs. 42% of the NSI children. Plasma p24 antigen levels tended to be higher in the NSI group with the difference approaching statistical significance ($P = 0.086$).

While the mean absolute CD4+ cell count was significantly lower in the SI group, after age-adjustment, expressing CD4+ cell count as a percentage of the median CD4+ cell count in healthy children of various age groups, counts tended to be lower in the SI group, but the difference did not reach statistical significance.

Children with an SI virus were not more likely to carry a diagnosis of AIDS. In the NSI group, 49% of the children had a diagnosis of AIDS compared to only 25% of the SI group. Even after controlling for age, age-adjusted CD4+ cell count, p24 antigenemia, and length of antiviral therapy in a multivariate model, children with NSI viruses appeared to be more likely to carry a diagnosis of AIDS, but confidence limits were wide and included unity. There were no statistically significant differences in the frequencies of several clinical manifestations between the SI and NSI groups (Table I).

Prophylaxis for *Pneumocystis carinii* pneumonia (PCP) and therapy with antivirals might be expected to delay a diagnosis of AIDS even in the presence of SI viruses. The frequencies of PCP prophylaxis were very similar in the two groups. In the SI group, 87% of the children were on antiviral therapy at the time of sampling, the mean antiviral therapy duration in the SI group was 82 ± 16 weeks. In the NSI group, 61% were on antiviral therapy at the time of sampling with a mean therapy duration of 40 ± 7 weeks. The difference in therapy duration was statistically significant but this is likely to reflect, in large part, the age difference between the SI and NSI groups.

In this group of 48 children, those with NSI viruses were younger, had a higher rate of AIDS diagnoses, a

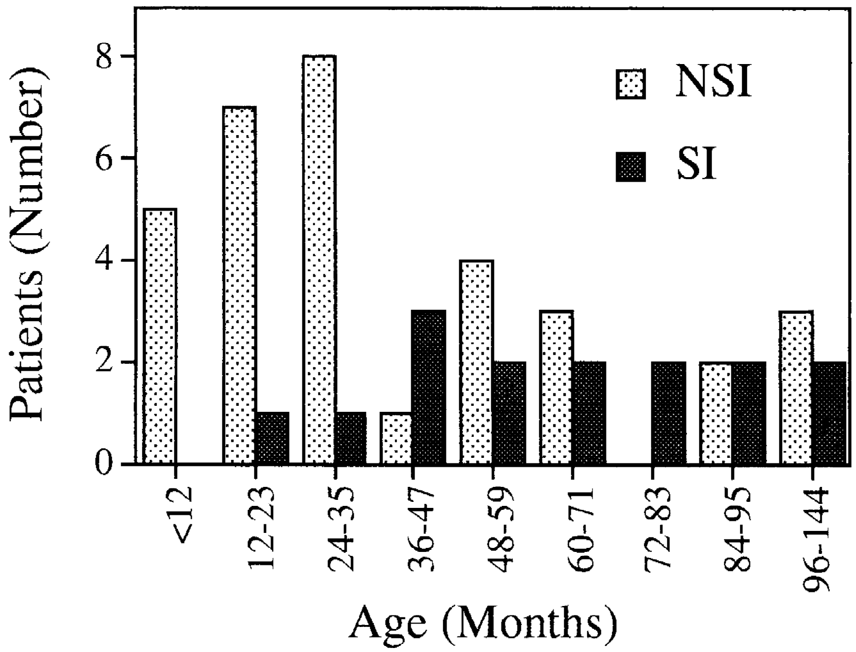


Fig. 1. Distribution of SI and NSI phenotypes as a function of age at sampling. Forty-eight perinatally-infected HIV-1 culture-positive children seen at the Central New Jersey Pediatric AIDS Program were assayed for the HIV-1 phenotype as detailed in Materials and Methods. Number of patients displaying each phenotype are shown for each age group.

higher rate of p24 positivity, and a higher mean p24 level than those with SI viruses. These differences appeared to be due, in large part, to a group of very young children with NSI viruses and an AIDS diagnosis. Twelve of 13 virus isolates from children less than 2 years old were NSI isolates. Seven of the 12 had a diagnosis of AIDS at the time of sampling and six died within 2 years of the sampling date. Of these six children, four died less than 4 months after an NSI virus isolation, two of the four within 1 month of NSI virus isolation.

When children under the age of 2 years were excluded from the analysis (Table II), the difference in age between subjects with SI and NSI viruses was not significant, plasma p24 levels were very similar, and p24 was detectable in 38% of the children with NSI viruses vs. 29% of the SI children. The subjects under 2 years of age from whom p24 data were available included nine children with NSI viruses; five of the nine had an AIDS diagnosis. These five children below the age of 2 years with an NSI virus and an AIDS diagnosis

TABLE II. Clinical Characteristics of Children With NSI vs. SI Viruses above 2 Years of Age

| | SI | NSI | p Value |
|---|---|---|---|
| Number of patients | 14 | 21 | |
| Age (mean in months ± SE) | 68 ± 8 | 57 ± 7 | 0.225[c] |
| CD4+ Count (cells/mm$^3$ blood, mean ± SE) | | | |
| Absolute | 364 ± 112 | 561 ± 116 | 0.337[c] |
| % Median[a] | 25 ± 7 | 35 ± 7 | 0.567[c] |
| Plasma p24[b] | 46 ± 27 | 46 ± 15 | 0.281[c] |
| No. p24 Antigen Positive (%)[e] | 4 (29) | 8 (38) | 0.721[d] |
| Antiviral therapy (mean in weeks ± SE) | 84 ± 17 | 60 ± 10 | 0.182[c] |
| PCP prophylaxis at time of sampling (%) | 11 (79) | 16 (76) | 1.000[e] |
| AIDS Diagnosis (%) | 3 (21) | 9 (43) | 0.282[d] |
| Opportunistic Infection (%) | 2 (14) | 6 (29) | 0.431[d] |
| Encephalopathy (%) | 2 (14) | 7 (33) | 0.262[d] |
| Lymphoid Interstitial Pneumonitis (%) | 3 (21) | 5 (24) | 1.000[d] |
| Bacterial infection (%) | 1 (7) | 0 (0) | 0.400[d] |
| CMV infection (%) | 5 (36) | 7 (33) | 1.000[d] |

[a]Percentage of age-adjusted normal median CD4+ cell count was calculated for each patient, the mean of the values ± SE for the SI and NSI groups is shown.
[b]Plasma p24 data were obtained for 14 of the SI subjects and 20 of the NSI's.
[c]Wilcoxon Rank Sum Test.
[d]Fisher's Exact Test.
[e]Plasma p24 level greater than 30 pg/ml.

had a very high mean plasma p24 value (161 ± 55 pg/ml).

CD4+ cell counts were very similar in the two groups when children under the age of 2 years were excluded. Among children below the age of 2 years, the group with an AIDS diagnosis had a much lower age-adjusted CD4+ cell count than the non-AIDS group. The one child with an SI phenotype below the age of 2 had an extremely low CD4+ cell count, 0.14% of the normal median age-adjusted count.

Among children above the age of 2 years the frequencies of PCP prophylaxis were very similar in the two groups, the difference in mean antiviral therapy duration between the two groups was no longer statistically significant and the number of children on therapy was very similar in the SI and NSI groups.

## Longitudinal Data

To study the stability of phenotypes over time, we examined sequential samples that were available from 14 of the patients. The mean follow-up time was 21 months. Five of the 14 had NSI isolates at their first sampling and remained NSI throughout the study. Another four had SI isolates throughout the study; these four remained clinically stable despite the presence of SI isolates for time periods of at least 16, 23, 28, and 31 months.

Three patients switched from an NSI phenotype to an SI phenotype at ages 5, 5.5, and 7 years, respectively. One of the three had very low CD4+ cell counts immediately prior to and after the switch and died 1 year after the phenotypic change. Another experienced a large decrease in CD4+ cell count after the switch (Table III).

Surprisingly, two patients switched from the SI phenotype to NSI at ages 2.5, and 10 years, respectively. To be sure that they did not represent sample mix-ups, these assays were repeated and the results were confirmed. One of these patients had three SI isolations followed by two NSI viruses, then another SI virus was isolated. The other patient also had multiple SI isolations, then two NSI virus isolations. In both of these patients, a decline in clinical course followed the appearance of the NSI phenotype. This was characterized by new opportunistic infections in both patients. During the time that SI viruses were isolated from the patients their CD4+ cell counts were low, but dropped to extremely low levels at the time of, or just prior to, isolation of the NSI virus (Table III). Plasma p24 levels were very low in one of the two patients at the time of the switch and declined after the switch in the other.

## DISCUSSION

Very few virulence factors have been found to be associated with HIV-1 infection. The syncytium-inducing phenotype is one such factor identified in adult patients [Tersmette et al., 1988, 1989; Koot et al., 1993a; Richman and Bozzette, 1994]. In our study of 48 perinatally-infected children we found that: 1) The SI phe-

TABLE III. Longitudinal Results of Pediatric Patients With HIV-1 Isolates That Switched Phenotypes

| Patient No. | Age (months) | Plasma p24 (pg/ml) | Phenotype | CD4 cell count Absolute | CD4 cell count % Median[b] | Clinical data (age in months) |
|---|---|---|---|---|---|---|
| 320 | | | | | | LIP (16) |
| | 23 | 0 | NSI | 1624 | 78 | Stable |
| | 35 | 1 | NSI | 1626 | 90 | Stable |
| | 58 | 1 | SI | 681 | 38 | Stable |
| | 64 | 2 | SI | 807 | 74.7 | Stable |
| 331 | | | | | | Encephalopathy (14) |
| | 66 | 173 | NSI | 59 | 3.2 | |
| | 69 | 117 | SI | 18 | 1.66 | |
| | 74 | 97 | SI | 21 | 1.94 | Death (81) |
| 347 | 77 | 11 | NSI | 603 | 55.8 | Stable |
| | 84 | 5 | SI | 180 | 16.6 | Stable |
| 346 | 13 | 137 | SI | 522 | 25.2 | Stable |
| | 19 | 43 | SI | 206 | 9.9 | Stable |
| | 25 | 13 | SI | 70 | 3.88 | Stable |
| | 30 | 4 | NSI | 8 | 0.44 | Cryptosporidiosis (31) |
| | 42 | 2 | NSI | 4 | 0.22 | |
| | 46 | 1 | SI | 4 | 0.22 | Death (50) |
| 209 | 104 | 158 | SI | 569 | 52.6 | |
| | 110 | 185 | SI | 479 | 44.3 | |
| | 115 | 152 | SI | 286 | 26.4 | |
| | 121 | 262 | SI | 16 | 1.48 | |
| | 123 | 131 | NSI | NA[c] | NA[c] | PCP (123) |
| | 127 | 36 | NSI | 12 | 1.1 | MAI (130) |

[a]Age in months.
[b]Percent of normal age-adjusted median CD4 cell count.
[c]NA, Not available.

notype of HIV-1 was no more likely to be associated with advanced disease stage than the NSI phenotype; 2) Some very young children progressed to AIDS and died without developing an SI isolate; 3) SI viruses were infrequent in children under the age of 2 years; and 4) A change from the SI to the NSI phenotype can occasionally occur in children.

We found that the SI phenotype did not occur preferentially in vertically-infected children with a diagnosis of AIDS. We did find a significant age difference between the SI and NSI groups with the children in the SI group being older than the NSI group. This was apparently due, in large part, to the number of children with NSI isolates below the age of 2 years. When children under 24 months were omitted from the analysis the age difference between children with SI vs. NSI isolates was no longer significant. A previous study by Spencer et al. [1994] found that, in children above the age of 2 years, older children were more likely to carry SI viruses and children above the age of 6 years carried SI viruses only. In our study, 21 patients above the age of 2 had an NSI phenotype compared to only 14 with an SI phenotype. Even above the age of 6, five patients carried NSI viruses. Such differences may be due to sampling errors or they may reflect characteristics of virus subpopulations existing in different geographical areas (Southern California vs. Central New Jersey).

In our study, seven of 12 children with NSI isolates below the age of 2 years had an AIDS diagnosis. These rapid progressors were responsible for the apparent tendency of children with NSI viruses to have higher plasma p24 levels and more AIDS diagnoses than children with SI viruses (Table I). Omitting children below the age of 2 years, thereby removing virtually all of the rapid progressors, resulted in less significant differences between the SI and NSI groups (Table II) especially in regard to age, age-adjusted CD4+ cell counts, plasma p24 antigenemia positivity, and mean p24 level.

The frequencies of PCP prophylaxis were very similar in the two groups, both in the overall analysis and in the analysis including only children above the age of 2 years. Therefore, the extent to which PCP prophylaxis might prevent or delay a diagnosis of AIDS was similar in both the SI and NSI groups and does not account for the low incidence of AIDS among the children with SI viruses.

The difference in mean antiviral therapy duration between the SI and NSI groups overall was statistically significant. However, the difference was probably largely due to the difference in age between the two groups, which was also significant, with older children having had a longer course of therapy. When children under the age of 2 years were removed from the analysis, the difference in mean antiviral therapy duration between the two groups was no longer statistically significant and the difference in age between the two groups was no longer statistically significant. Yet the incidence of AIDS in the SI and NSI groups remained essentially the same. These data indicate that the effect of antiviral therapy on the development of an AIDS diagnosis is probably very similar in the SI and NSI groups.

That children can progress quickly to AIDS and die without an SI isolate is evidenced by the fact that four of 12 children with NSI isolates below the age of 2 died within 4 months of their last NSI virus isolation. Of these, two died within 1 month of their last NSI isolation. It seems unlikely that their viruses converted to an SI phenotype immediately before death. Although adult patients often progress and die without ever having developed SI viruses [Koot et al., 1993b], disease progression generally occurs more slowly in adults with NSI viruses vs. those with SI viruses [Bozzette et al., 1993; Koot et al., 1993a].

Unlike previous studies, we did find one child below the age of 2 years who had an SI isolate. Virus isolates from the mothers of the children in this study were not available and it is not known whether the child was infected with an SI isolate or developed an SI phenotype during the first year of life.

Analysis of multiple sequential samples from 14 children indicated that a switch from the NSI to the SI phenotype can sometimes occur with a concomitant decrease in CD4 cell count as has been reported in adults [Tersmette et al., 1988, 1989; Koot et al., 1993a; Richman and Bozzette, 1994]. Two of the three patients that converted from NSI to SI appeared to experience a sharp decline in CD4 cell count after the conversion, whereas a third patient did not appear to experience the decline and remains stable both immunologically and clinically after the switch. Continued follow-up of our NSI patients will help determine whether the NSI to SI switch generally carries a poor prognosis in children.

We observed two cases of conversion from the SI to the NSI phenotype. An SI virus was again isolated from one of the patients after two NSI isolations. This fluctuation in phenotype has been observed in at least one adult patient [Karlsson et al., 1994]. Unfortunately, conversion from SI to NSI was accompanied by a decline in clinical status in the pediatric patients in our study. Both children experienced a decline in CD4+ cell count (Table II) and new opportunistic infections. The switch from the SI phenotype to NSI when CD4 cell counts were already low did not appear to be advantageous in these children.

What would explain a switch from SI to NSI in these two children? In adults acutely infected with primarily SI viruses the initial immune response may focus on highly cytopathic SI variants, thus clearing the SI viruses and setting the stage for a slower disease course mediated by the slower-replicating NSI variants. It seems unlikely that the switch was mediated by a host immune response in our patients since both had SI viruses for at least 1 year prior to the switch. In fact, the “switch” from SI to NSI may not be a switch at all. If these patients had a high NSI to SI virus ratio and a high viral burden, then blood samples used to culture the virus would usually contain SI viruses, as well as

NSI viruses, and the viral isolate would be scored as SI. If the viral burden dropped, perhaps after a change in antiretroviral therapy, some blood samples might only contain NSI viruses and the isolate would be scored as NSI. Our two patients had no change in their therapy (zidovudine monotherapy) for at least 1 year prior to the switch. Another possibility involves HIV-1 co-receptor usage. Although the SI phenotype and co-receptor usage are usually linked, this may not be true in all cases. Thus, some NSI viruses might be capable of utilizing the same co-receptor as SI viruses and may replicate to the same high levels but without inducing syncytia on MT-2 cells. This might lead to a very high ratio of NSI to SI virus and the SI viruses might be excluded during blood sampling. Alternatively, highly cytopathic SI viruses may have depleted circulating CD4+ T-cells to a point where they could no longer sustain the SI population in these children. Monocytes and/or macrophages, infected earlier in the course of infection with NSI viruses, might then have provided the viruses that grew when the patients were cultured. Recovery of an SI or NSI virus from these patients may occur at random depending on the viruses captured in the blood sample used to isolate the virus. This sampling effect may explain why an SI virus was again isolated from one of these patients after two NSI viruses had been recovered earlier in his course.

Some children, despite carrying SI viruses, can remain stable for prolonged periods of time, an observation consistent with the findings of Gupta et al. [1993] and Spencer et al. [1994]. Persistence of SI viruses in pediatric patients with stable disease seems surprising in light of the ample evidence that the SI phenotype is associated with advanced disease in adults [Koot et al., 1993a; Richman and Bozzette, 1994; Tersmette et al., 1988, 1989]. In adults, changes that result in the SI phenotype also appear to allow utilization of the CXCR4 molecule as a second receptor on CD4+ T-cells [Deng et al., 1996]. In children, these two phenotypes may not be linked. Variations in the expression of CXCR4 or its ligand could affect the ability of the virus to enter CD4+ T-cells.

In summary, children in our study displayed a biphasic pattern of HIV-1 progression similar to that found previously [Spencer et al., 1994]. Many children below the age of 2 years carried NSI viruses, an AIDS diagnosis, low CD4+ cell counts, and high plasma p24 levels. These rapid progressors do not appear to develop SI viruses even at points very close to death. Furthermore, in children over 2 years of age, infection with SI viruses did not increase the likelihood of an AIDS diagnosis. Children with SI viruses were very similar to the NSI patients in all categories including age-adjusted CD4+ cell counts and p24 antigenemia. We noted that some children remained stable for 2 years or more despite carrying SI viruses and that some children beyond the age of 6 years carried NSI viruses. In some children, switches from NSI to the SI phenotype and from SI to NSI did occur, the latter occurring at times when CD4+ cell counts were very low. The more advanced clinical stage associated with the SI phenotype in adults may not apply to children with HIV-1 infection.

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